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Exploring the Physiological Traits of Eggplant (*Solanum melongena* L.) Cultivated in a Sandponics Growing System

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Sandponics is an advanced system that promotes food security. It involves growing plants and fish together in a recycled ecosystem that uses natural bacterial cycles to use fish waste as plant nutrition. Eggplant is considered one of the most important crops known for its nutritional benefits. Eggplant (French Flomorin species), were grown in Sandponics panels and regular ground, where five plants were randomly selected from each experimental unit in the vegetative growth stage, physiological indicators and antioxidant were recorded. The results showed that there was a significant difference for water quality in total chlorophyll content, showing higher levels in plants irrigated with fish-free water than those with fish water, and there were no significant differences for water quality in chlorophyll A concentration and chlorophyll B concentration. The results also indicate that there is a significant difference between water quality for the concentration of glutathione, as the highest concentration was recorded in fruits irrigated with fish water compared to fruits irrigated with fish-free water. While there is a significant difference between the quality of water for the concentration of proline, as the highest concentration was recorded in fruits irrigated with fish-free water compared to fruits irrigated with fish wat. There are significant differences in water quality for nitrogen, phosphorus, potassium, and iron. The average concentrations are higher when watered with fish water, compared to the average concentrations when irrigated with fish-free water.

Keywords: Cultivation systems, vegetables, physiological indicators, antioxidants, microelements, macro-elements.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a crop of Solanaceae family, self-pollinating plant and its cultivation is widespread in various countries of the world as it is a light-neutral plant. Nutritionally, eggplant contains relatively low amounts of proteins, fats, soluble carbohydrates, and important vitamins and minerals. However, it is very rich in fibers. (Alam and Salimullah,2021). Sandponics is a sustainable crop production method that uses sand as a primary medium for mechanical filtration, bio-filter, and crop growing medium (Makokha *et al*., 2019). This method is characterized by the production of completely organic plants that do not need any inorganic fertilizers because the plants obtain their fertilizer from the conversion of ammonia in fish water into nitrates, where the sand acts as a filter, and this contributes to increasing production efficiency and reducing cost (El-Nemr *et al*., 2012).

One of the important physiological characteristics that must be present in plants is the proportions of chlorophyll, proline, glutathione, and anthocyanins. The importance of chlorophyll does not lie in giving the plant the green color. Rather, it plays an important role in the process of photosynthesis, as the chloroplasts carry out this process inside the plant's leaves. Chlorophyll a and chlorophyll b are two types of chlorophyll pigments that participate in the process of photosynthesis in plants and algae (Scheer, 2022). Where chlorophyll a represents the primary photosynthetic pigment that absorbs energy from wavelengths of blue-violet and orange-red light, while chlorophyll b is an accessory pigment that absorbs energy from wavelengths of green light (Pérez-Gálvez *et al*., 2020). Proline is one of the most important essential amino acids involved in the formation of proteins. The accumulation of proline within the plant may occur as a response to its adaptation or sensitivity to a certain stress (Bates *et al*., 1973). Glutathione is considered an antioxidant capable of preventing damage to important cellular components caused by reactive oxygen species, free radicals, peroxides, lipid peroxides, and heavy metals (Minich, 2019; Silvagno, 2020). As for anthocyanins, they are water-soluble pigments that have physiological importance in plants and can be analyzed in physiological fluids using advanced analytical methods

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(Ayvaz, 2022). The basic macro- and micronutrients required for optimal plant growth also include nitrogen, phosphorus, potassium, calcium, iron, zinc, copper, and manganese (Thorarinsdottir *et al*., 2015). Nitrogen is an essential element that affects plant growth and crop productivity. It is a mobile element in plants as a component of amino acids, proteins, coenzymes, nucleic acids, and chlorophyll. Plants absorb nitrogen in the form of nitrate ($NO3^{-1}$) or ammonia ($NH4^{+1}$) (Jones, 2016).

Phosphorus considered one of the components of ATP, nucleic acids, phospholipids, and some coenzymes, and its deficiency can significantly slow the growth of plants (Jones, 2016). Potassium is also an element that stimulates the process of photosynthesis, as there is a decrease in the ability of plant leaves to carry out the process of photosynthesis when the level of potassium in the plant decreases due to its effect in building the compound ATP, which is the main carrier of energy in the plant and a store for it to represent CO_2. Potassium also affects the synthesis of sugars and proteins and the reduction of nitrates, and increases the plant's resistance to pests, diseases and fungi (Marschner, 1995).

MATERIALS AND METHODS

For the purpose of studying the use of sandponic system for growing vegetables to address water scarcity, a field experiment was conducted in the greenhouse in Baladruz, Imam Askar district, located east of Diyala province, during Winter 2023.

***Experimental Design*:** The field, which about 120 m^2 area, was prepared in terms of plowing, smoothing and leveling the soil. The sandponics planting area was designed in the form of panels built with bricks with three panels (T1, T2, T3), 3m long, 1m wide, and 40cm high. Each panel was covered with an insulating nylon, (2) plastic irrigation tubes (1.5) inches in diameter were placed on both sides of each panel, that were dissected and wrapped with gauze.

Two basins were prepared, (2) m length, (1) m width, and (1) m height, and filled with (1,500) liters of water, the water source being the Roz River. Which originates from Lake Hamrin, Diyala province, the same amount of water was filled in other two basins, i.e. 1500 liters in each basin, which was used in sandponic panels and the ordinary land, and (3) panels were prepared in field (T4, T5, T6), 3 m long and 1 m wide.

***Planting and Cultivation*:** One hundred common carp species of fish were brought from one of the private farms in Nahrawan, Baghdad province, and placed in system's tank. The aquarium was prepared in terms of oxygen, and a third tank was prepared, 1 m length, 1 m width and 50 cm height. It is used to collect water from watering sandponic panels and return it again to the aquarium. Then fine sand was placed in sandponic panels, and washed several times before planting. Eggplant, (French variety Flumorin), was planted on 12/25/2022 in sandponic panels and in regular ground. A distance of 23 cm was left between each plant and another, where (24) plants were planted in each panel, in both sandponic and regular panels, that is, a total of (72) plants in sandponic panels, and the same number in the ground cultivation.

MEASUREMENT TECHNIQUES

***Measurement of the relative content of chlorophyll in leaves (SPAD)*:** The percentage of chlorophyll in the leaves was measured during the flowering stage by taking three plants at random, and then the rate in each experimental unit was extracted using a spad-502 chlorophyll meter.

***Determination of chlorophyll (A, B)*:** The total chlorophyll pigment in the leaves is estimated by taking the fourth leaf from the growing apex (Al-Sahhaf, 1989) of each plant in each treatment, washing it well with water and drying it airily. Then, 1 gm is taken from each sample, After that, the leaves tissue is cut to facilitate the extraction process, then 10 ml of acetone, 85% concentration, and 5 gm of sodium carbonate are added to it to prevent the breakdown of chlorophyll pigment. Then the leaf tissue was crushed with acetone in a ceramic mortar and the dye solution was isolated using filter paper. The UV-visible spectrophotometer is used to measure the optical absorbance of pigments at two wavelengths (645 and 663 nm), and the amount of total chlorophyll pigment is calculated (mg pigment g^{-1} tissue soft paper) (Goodwin, 1976). Chlorophyll is estimated after the spectrophotometer reading of plant samples is measured by applying the following equations:

$$\text{Total chlorophyll} = \{20.2\times D(645)\} + \{8.02\times D(663)\}(v/w\times1000)$$

$$ChI.a=(12.7(D633)-2.69(D649))\times V/(1000\times w).$$

$$ChI.b=(22.9(D645)-4.68(D663))\times/(1000\times w).$$

Where, D: Optical absorption, D(663) = optical absorption reading at a wavelength of 663 nm, D(645) = optical absorption reading at a wavelength of 645 nm, V = final volume extracted (10 ml), W = weight of paper fabric (1gm).

Determination of proline

***Extraction process*:** Proline was extracted according to the method presented by Dahl Lassen (2018), 3 gm of sample was taken and placed in 25 ml volumetric vial, and 25 ml of 1M hydrochloric acid at 55°C for 3 hrs. The sample was dried using a rotary evaporator, then 5ml of sodium citrate, pH 2.2, was added. The sample was filtered using a 0.45um plastic filter and taken to the device to perform the injection.

***Derivation process*:** Take 1 ml of the extracted sample and add 200 µl of orthophthalein aldehyde (OPA) 5%. The sample is shaken for 2 min, then 100 µl is taken from the last mixture were injected into the Amino acid analyzes device. The examination was conducted in the laboratories of the Ministry of Science and Technology/ Department of Environment and Water using high-performance liquid chromatography (HPLC model SYKAM- German). The method presented by

(Scriver CR, 2001), where the carrier phase was used, which consists of methanol: acetonitrile: 5% formic acid in ratios of 20:60:20 at a flow rate of 1 ml/min. A C18-ODS separation column was used to separate amino acids, while a fluorescence detector was used to detect amino acids with longer wave lengths: Ex = 445 nm, Em = 465 nm and the Clarity 2015 program was used to analyze amino acids.

***Calibration curve*:** 0.1 g of a mixture of high purity amino acids (99.9%) was dissolved in non-ionic water and transferred to a 250 ml conical flask. The volume was completed to the mark, where its concentration became 250 ppm. Using the dilution law, the calibration curve concentrations injected into the device were prepared.

Determine the total anthocyanin content

***Sample extraction*:** Transfer 1 gm of each sample to a test tube, then add 3 ml of methanol and mix by vortex for 30 sec. The test tubes were covered and placed in a water bath at 60°C for 20 min. then mixed by vortex twice during incubation, so that methanol layer in each test tube was subsequently separated by centrifuging (10,000 rpm) for 10 min and the dissolved supernatant was transferred to a 10 ml volumetric flask. The remainder was mixed again with 3 ml of methanol, so that the supernatant was separated as previously described and combined with the previous supernatant. The volume of the tube containing the supernatant was adjusted to 10 ml, so that the extracted solution was kept at 0°C until the analysis was performed. The total anthocyanin content (TAC) was determined by pH-differential anthocyanin pigments, which undergo reversible structural transformations with a change in pH that appear through a different absorption spectrum. The colored form of oxonium predominates at pH 1.0 and the colorless hemiketal form prevails at pH 4.5. The pH-differential method is based on this reaction and allows accurate and rapid measurement of total anthocyanins, even in the presence of degraded pigments, Polymers and other interfering compounds. One milliliter of the extracted solution was transferred to a 10 ml volumetric flask to prepare two dilutions of the sample, one adjusting the volume using potassium chloride buffer, pH equal to 1.0, and the other with sodium acetate buffer, pH = 4.5, dilute each, then leave the dilutions to equilibrate for 15 minutes. Then the absorbance of each dilution was measured at 510 and 700 nm (to correct haze), against an empty cell filled with distilled water. All measurements should be made between 15 min and 1 hr after sample preparation, as longer waiting periods tend to increase observed readings.

Absorbance is read against water blanks. The samples to be measured must be clear and do not contain fog or sediment. However, some colloidal materials may be suspended in the sample, causing light scattering and a cloudy appearance (haze). This light scattering needs to be corrected by reading at a wavelength where no absorption by the sample occurs, in other words at a wavelength of 700 nm.

The absorbance of the diluted sample (A) is calculated through the following equation:

$$A = (A510 – A700)pH\ 1.0 – (A510 – A700)pH\ 4.5$$

The concentration of the monopigment anthocyanin in the original sample is calculated using the following equation:

Anthocyanin pigment (mg/Kg) = (A x MW x DF x 1000) / (εx1)

It was converted to mg of total anthocyanin content/100 g sample. Where MW is the molecular weight, DF is the dilution factor, and ε is the molar absorbance, and the dye content is calculated as cyanidin-3-glucoside, where MW equals 449.2 and ε = 26900.

***Estimation of elements in plants*:** The elements were estimated in collected plant samples, dried, and grounded. The powder was digested using the acid digestion or wet washing method according APHA (2017), where 3 gm of the desired sample powder were placed and digested in 25 ml (Griffin brewer) cup. Then 3 ml of concentrated perchloric acid solution was added and the cup was covered using a glass watch and heated on hot plate quietly. The temperature was gradually raised to complete the digestion process. When the mixture reaches the dry stage, the cup is left to cool. 3 ml of concentrated nitric acid solution is added again, and the cup is covered and continues heating until the digestion process is completed. We obtain a mixture that is formed in a clear form and is colored with a light color called (light colored digestate). The evaporation process is carried out until it approaches to the drying stage, 5 ml of diluted hydrochloric acid solution with water in a ratio of 1:1 was added, then we carried out the heating process. This is to dissolve the remaining sample after the digestion process, then distilled water is added, and the filtration process is done to get rid of the remaining and insoluble materials, and the size of the solution is adjusted according to the expected concentration in the samples to a volume of 100 ml or 50 ml or less, as the sample becomes ready for analysis. The absorbance of these digested samples was measured using an atomic absorption device (SHEMADZU AA 7000).

***Estimation of phosphorus percentage (%)*:** The plant's phosphorus content was estimated by taking (0.5 g) of the dried grounded sample and dissolved with (5 ml) sulfuric acid, (2 ml) perchloric acid, and molybdenum was used. Aluminum and ascorbic acid (colorimetric method) were then measured using a spectrophotometer (model Shimadzu - 1650) at a wavelength of (700 nm).

***Estimation of nitrogen percentage*:** The Kjeldahl method was used to estimate the percentage of nitrogen in samples, based on Van Dijck *et al*. (2000) method by taking 50 gm in a beaker. 5 ml of concentrated sulfuric acid was added to the sample, and an appropriate amount of the mixture of potassium sulphate and copper sulphate was added. The digestion process was carried out by heating the contents, and after the completion of the digestion, the mixture was transformed into a clear liquid with a pale blue color. The

liquid was transferred to the distillation flask of the Kjeldahl device, which contains a concentrated solution (40%) of sodium hydroxide and connected to a condensing distillation flask that ends with a test tube that is immersed in a receiving flask containing a known volume of boric acid (20%), added a drops of methyl red indicator and bromocresol blue dye. Then the estimation flask is heated until the amount of distilled liquid collected in the flask reaches about (25 ml), then the collected liquid is flushed with (0.1) standard hydrochloric acid and a control solution is prepared (Planck) of the chemicals above, except for the sample. The protein percentage is calculated according to the following equation:
Nitrogen percentage % = volume of HCl consumed x standard x 0.014/sample weight x 100

RESULTS

***Chlorophyll (SPAD unit)*:** Table 1 shows that there is a significant difference for water quality in terms of total chlorophyll content, with a higher average with water without fish, reaching 56.900 compared to fish water, with a lower average 51.522 SPAD unit. The results of the same table also showed that there were no significant differences for water quality in the concentration of chlorophyll A and the concentration of chlorophyll B.

Table 1. The effect of water type on leaf pigments in eggplant (SPAD unit).

Water type	Pigments		
	Total chlorophyll	Chlorophyll A	Chlorophyll B
Water with fish	51.52 B	0.207	0.067
Water without fish	56.90 A	0.200	0.077
Average	54.21	0.203	0.072
Duncan's test	Significant	Not Significant	Not Significant

***Antioxidants (Glutathione, Proline, Anthocyanin)*:** The results of Table 2 indicated a significant difference between water quality for glutathione concentration, as the highest concentration was recorded in fruits irrigated with fish water 32.400 units, compared to fruits irrigated with water without fish, 25.798 units.
The results of Table 2 also showed that there was a significant difference between water quality for the concentration of proline, as the highest concentration was recorded in fruits irrigated with water without fish, 6.351 units, compared to fruits irrigated with fish water, 3.787 units.
For the anthocyanin pigment, Table 2 showed that there was a significant difference between the quality of water, as the highest concentration was recorded in fruits irrigated with fish water, 1.188 units, compared to the concentration of anthocyanin pigment in fruits irrigated with fish free water without, which was recorded 0.937 units.

Table 2. The effect of water type on antioxidants in eggplant fruits.

Water type	Antioxidants		
	Glutathione	Proline	Anthocyanin
Fish Water	32.400 A	3.787 B	1.188 A
Water without fish	25.798 B	6.351 A	0.937 B
Average	29.099	5.069	1.063
Duncan's test	Significant	Significant	Significant

***Estimation of elements in plants*:** It includes the basic macro- and micro-nutrients necessary for plant growth, such as nitrogen, phosphorus, potassium, calcium, iron, zinc, copper, and manganese, respectively. Iron is usually deficient in fish waste and may require supplementation when symptoms appear early in the plant (Thorarinsdottir *et al*., 2015).
***Estimating the elements in leaves*:** The results of Table 3 indicate that there are significant differences in water quality for nitrogen, phosphorus, potassium, and iron, with higher concentrations when irrigated with fish water, reaching 0.136, 0.077, 1.405, and 0.073 sequentially, compared to their average concentrations when irrigated with water without fish, which were 0.101, 0.030, 1.054, 0.045 respectively.

Table 3. The effect of water type on the concentration of elements in eggplant leaves.

Water type	Average of Elements			
	Nitrogen	Phosphorus	Potassium	Iron
Fish Water	0.136 A	0.077 A	1.405 A	0.073 A
Water without fish	0.101 B	0.030 B	1.054 B	0.045 B
Average	0.118	0.053	1.230	0.059
Duncan's test	Significant	Significant	Significant	Significant

DISCUSSION AND IMPLICATIONS

The study found a significantly higher total chlorophyll content in eggplants irrigated with water without fish compared to those with fish water. This could be attributed to the adverse effects of ammonia or other compounds present in fish water, potentially impacting chlorophyll synthesis and chloroplast stability, leading to reduced photosynthesis efficiency.
Chlorophyll A and chlorophyll B were equal in both irrigation methods, this agreed with the results of Çekin (2023), as the average chlorophyll A was relatively equal in all agricultural systems. The reason for the decrease in chlorophyll in the leaves of eggplant irrigated with fish water may be due to the accumulation of ammonium ions (NH_4), which in turn leads to the destruction of chloroplast and the destruction of chlorophyll, which leads to a decrease in the efficiency of photosynthesis (Saqr, 2012).
Glutathione was higher in fruits irrigated with fish water, this possibly due to an increase in oxidative stress or biological stimulation from compounds in fish water. There could be

several reasons for increasing the percentage of glutathione in plants, including the effect of ammonia, as fish water contains compounds such as ammonia (NH_3), which result from the metabolism of proteins in fish. Plants utilize these compounds as a source of nitrogen, which is a component of glutathione (Wang *et al*., 2010). Another reason is biological stimulation, i.e. the presence of ammonia and organic materials in fish water stimulates the activity of bacteria and fungi in the roots, which leads to increased glutathione production (Dubreuil-Maurizi and Poinssot, 2012). Glutathione works to regulate oxidative stress and maintain cell stability. It acts as an antioxidant, helping protect cells from damage resulting from free radicals and oxidation. It also helps plants adapt to surrounding environmental conditions, such as high temperatures, drought, and pollutants. It plays a role in regulating plant growth and development, including the formation of buds, leaves, and fruits (Noctor *et al*., 2011).

Proline was higher in fruits irrigated with water without fish, likely as a response to water stress, aiding in plant tissue protection., as it contributes to binding and restricting the toxic elements absorbed under these conditions. The increase and accumulation of proline is due to a decrease in its oxidation and an increase in proline catabolism and its transformation into amino acids, including proline (Saqr, 2012). Anthocyanin was higher in fruits irrigated with fish water, which could be related to the specific stress responses or nutrient availability in the fish water.

Elemental Concentration (Nitrogen, Phosphorus, Potassium, Iron): All these elements showed higher concentrations in eggplant leaves irrigated with fish water. This indicates that fish water provides a rich source of these nutrients, essential for plant growth. Fish waste is particularly noted for its nitrogen and phosphorus content, which are critical for plant development and photosynthesis.

The results of this study agreed to Makokha (2019) results, where the average concentration of nitrogen and phosphorus was higher in the sandponic system. It differed with the results of the same researcher regarding the element potassium, as its average concentration was lower in the sandponic system. The results agree with Afolabi (2020), where the average iron concentration was less than 0.2. Of all the chemical nutrients exchanged in the sandponic system, nitrogen and phosphorus are the most important (Lam *et al*., 2015). According to some researchers (Schmautz *et al*., 2016: Kimera *et al*., 2021), the main sources of nutrients in sandponic systems are the added water source (which contains Mg, Ca, S), Uneaten fish feed and fish waste. Fish waste contains a significant level of nitrogen and phosphorus, although smaller amounts of other trace elements are present. Both macro- and microelements are vital for plant growth in varying amounts (Somerville *et al*., 2014; Mzoughi *et al*., 2019). Nitrogen, phosphorus and potassium also have a significant impact in increasing the effectiveness of plants photosynthesis and respiration, as they are involved in the synthesis of the nucleic acids DNA and RNA necessary for cell division and thus increasing the height of plants (Al-Sahhaf *et al*., 1992 ; Chowdhury *et al*., 2002 ' Imma *et al*., 2006).

In addition to the role of phosphorus in increasing the number of microorganisms in the soil and increasing their activity, which increases the mineralization and readiness of the elements in the soil and then in the plants, Besides improving the composition, maintaining the percentage of moisture, and increasing aeration of the soil, which provides a suitable environment for root growth and its impact on Good absorption of elements (Diver *et al*., 1999). Or irrigation water with fish may provide phosphorus through the formation of chelating compounds that protect phosphorus from fixation and work to prolong its availability throughout the plant's growth period (Edmeades, 2003;Laboski and Lamp, 2003). The increase in phosphorus readiness may be due to the increase in living mass as a result of irrigation with fish water, and this leads to an increase in the released CO_2, which forms carbonic acid (H_2CO_3) after dissolving in water, which leads to a decrease in the pH of the soil and an increase in the solubility of phosphate compounds. deposited, which leads to the release of phosphorus and increases its readiness for the plant (Herencia *et al*., 2006).

The presence of fish waste in the irrigation water seems to influence nutrient availability, particularly nitrogen and phosphorus, which are crucial for plant growth. The findings align with other research in the field, supporting the idea that aquaponic or sandponic systems (where fish and plants are cultivated together) can be efficient in nutrient recycling and utilization. The study also touches on the complex interactions between plants and their environment, especially in terms of stress response (glutathione and proline levels) and nutrient uptake. Finally, the impact on chlorophyll content suggests a need for careful management of nutrient levels and possibly the mitigation of harmful compounds in fish water to avoid negative effects on photosynthesis.

Conclusions: The study highlights significant differences in the biochemical composition of eggplant when irrigated with water containing fish waste versus regular water. This suggests potential benefits and challenges of using fish-enriched water in plant cultivation, particularly in systems like aquaponics. The higher nutrient content in fish water can be advantageous, but the potential stress or adverse effects on photosynthesis need to be managed.

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Standard measurement units and their abbreviations

Full name	***Symbol***		
Centimeter	Cm.	Gram	gm
Meter	m	Nanometer	nm
Minute	min	Hour	hr
Milliliter	ml	Round per minute	rpm
Optical absorbance	D		